REMARKS

Status of Claims

Claims 1-3, 5-11, and 21-59 are pending in this application and were examined. Claims 12-13 are pending but were withdrawn from consideration as being drawn to non-elected inventions.

This paper amends claims 1 and 3. Claims 1-3, 5-11, and 21-59 are currently under examination.

The Office Action

Claims 1-3, 5-11, and 21-59 stand rejected under 35 U.S.C. § 103 as obvious over Hafez et al. (*Biophysical Journal*, 79: 1438-1446, 2000; "Hafez") in view of Huang (U.S. Patent 5,283,122; "Huang") or Lishko et al. (U.S. Patent 5,753,263; "Lishko"). Claims 1-3, 5-11, and 21-59 stand rejected under 35 U.S.C. § 103(a) as obvious over Deshmukh et al. (U.S. Patent 6,258,792; "Deshmukh") alone or, in the alternative, over Hafez in view of Deshmukh.

Rejections Under 35 U.S.C. § 103

I. The Claimed Invention

The invention, as currently claimed, is an amphoteric liposome that contains an anionic lipid, a cationic lipid, and a neutral lipid that is stable both at a low pH and a neutral pH. The liposomes of this invention are fusogenic at intermediate pH values. These liposomes, therefore, have a "stability trough" at intermediate pH values.

This stability profile provides several advantages to the liposomes. First, the instant liposomes are stable at low pH where they adopt a cationic charge. This provides optimal conditions for loading polyanionic active ingredients such as nucleic acids. Second, the instant liposomes are also stable at a higher pH (i.e., neutral pH) where they adopt an anionic charge. Neutral pH values and anionic liposomes are optimal for the washing and storage of liposomal formulations as well as for intravenous administration. Anionic liposomes, when injected intravenously, are more compatible with serum and blood components and do not form large aggregates. Third, the "stability trough" at slightly acidic pH in which the liposomes are

maximally fusogenic corresponds to the acidic conditions often found in endocytosed vesicles, tumors, and at sites of inflammation and other pathological conditions.

In sum, the instant liposomes display a complex profile of pH-dependent fusogenicity not observed in the liposomes of the prior art.

II. Applicable Legal Standard For Obviousness

In order to make a *prima facie* case of obviousness, the Examiner must demonstrate that the prior art (i) teaches or suggests every claim limitation, (ii) provides a motivation to combine (or modify) the teachings of the selected references, and (iii) provides a reasonable expectation of success. M.P.E.P. § 2143.

The Examiner must demonstrate that the prior art specifically provides a motivation to combine the teachings of the selected references. The fact that the modification of the prior art to arrive at the claimed invention is within the capabilities of the skilled artisan in not sufficient by itself to provide a motivation to combine references. In re Kotzab, 217 F.3d 1365, 55 USPQ2d 1313 (Fed. Cir. 2000); Al-Site Corp. v. VSI Int'l Inc., 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999).

It is well established that prior art references are not properly combined, and fail to establish a *prima facie* case of obviousness, if their combination or modification renders the device inoperable for its intended purpose. In re Gordon, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984) (not obvious to turn the prior art device upside down because it would render the device inoperable); Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 20 U.S.P.Q.2d 1746 (Fed. Cir. 1991).

In order to avoid the inappropriate use of a hindsight analysis, the courts have repeatedly cautioned that the teaching or suggestion to make the claimed combination must be found in the prior art, not in applicant's disclosure. See, In re Dembiczak, 175 F.3d 944, 50 USPQ2d 1614 (Fed. Cir. 1999). "It is impermissible to first ascertain factually what [applicants] did and then view the prior art in such a manner as to select from the random facts of art only those which may be modified and then utilized to reconstruct appellants invention from such prior art." Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985).

III. Rejection over Hafez in View of Huang and Lishko

Claims 1-3, 5-11, and 21-59 stand rejected under 35 U.S.C. § 103(a) as obvious over Hafez in view of either Huang or Lishko. Specifically, the Examiner asserts that Hafez teaches liposomes, containing CHEMS (an anionic lipid) and DODAC (a cationic lipid), that have a pH_f 4.0 to 6.7, but lacking the inclusion of a neutral lipid such as cholesterol or phosphatidylcholine (PC). The Examiner further asserts that Huang discloses that the inclusion of cholesterol into pH-sensitive liposomes reduces the leakage of liposomal contents and that Lishko discloses the inclusion of PC or cholesterol into pH-sensitive liposomes. The Examiner concludes, therefore, that it is obvious to modify the pH-sensitive liposomes of Hafez by adding a neutral lipid, as taught by either Huang or Lishko, to arrive at the claimed invention. Applicants respectfully disagree.

A. The Deficiency of Hafez

The liposomes of the present invention are fundamentally different from those of Hafez. As discussed above, the liposomes, as currently claimed, are amphoteric and are stable enough at a low pH that they are capable of being loaded with polyanions such as nucleic acids and are also stable at a neutral pH, suitable for storage and administration.

By contrast, the CHEMS/DODAC liposomes of Hafez are shown to be increasingly fusogenic as the pH is lowered (see Figures 2A). The other type of Hafez liposomes—DOPA/DC-Chol (>1.6:1)—were prepared (and stable) at a low pH, but became increasingly fusogenic as the pH was raised (see Figure 4A). In an alternative formulation, the DOPA/DC-Chol (1.1-1.6:1) liposomes were prepared at a neutral pH at were demonstrated to become increasingly fusogenic as the pH was lowered (see p. 1441, right column; data not shown).

Both the CHEMS/DODAC and DOPA/DC-Chol (1.1-1.6:1) liposomes of Hafez, prepared at a neutral pH, may be useful for storage and administration but they are not easily loaded with anionic therapeutics. The DOPA/DC-Chol (>1.6:1) liposomes prepared at an acidic pH may be loaded with anionic therapeutics are not easily stored or administered at a neutral pH. Nowhere does Hafez teach or suggest how to modify the liposomes to be stable both at a low pH and a neutral pH. Thus, the Hafez liposomes have substantially different properties than those of

the present invention. For this reason alone, Hafez fails as a primary reference to support an obviousness rejection.

B. Huang and Lishko Do Not Provide What Hafez Lacks

Neither Huang nor Lishko provide what Hafez lacks. Huang discloses liposome having mixtures of anionic and neutral lipids. These liposomes, like those of Hafez, become increasingly fusogenic as the pH is lowered (see, for example Figure 2 with col. 5, ll. 22-26, and Figure 3 with col. 5, ll. 40-50). Thus, Huang does not provide a liposome that is stable both at a neutral pH and an acidic pH while being fusogenic at an intermediate pH as in the current invention.

Likewise, Lishko teaches liposomal formulations of anionic and neutral lipids. The Lishko liposomes comprise one or more of PC, EPC, DOPC, DPPC, PE, DOPE and cholesterol, combined with one or more lipids to form a pH-sensitive liposome (col. 15, ll. 13-17). However, all of the combinations suggested by Lishko would be fusogenic at a low pH when the liposomes are essentially uncharged. There is nothing in Lishko to teach or suggest to the artisan the desirability or the methodology for making a liposome that is stable at a low pH.

Therefore, nothing in either Huang or Lishko teaches the artisan how to make an amphoteric liposome that is stable at a low pH. Thus, the combination of Huang or Lishko with Hafez does not result in the instant invention. Accordingly, this rejection should be withdrawn and such action is respectfully requested.

IV. Rejection over Deshmukh

Claims 1-3, 5-11, and 21-59 stand rejected under 35 U.S.C. § 103(a) as obvious over Deshmukh. Specifically, the Examiner states that Deshmukh teaches cationic liposomes having a cationic, an anionic, and a neutral lipid. Office Action at p. 6, ll. 2-3. The Examiner asserts that an artisan is motivated, based on the teachings of Deshmukh, to vary the amounts of cationic and anionic lipids to obtain a liposome with the desired net positive or negative charge at physiological pH, depending upon the use of the liposome to arrive at the presently claimed invention. Office Action at p. 6, ll. 11-14. Applicants respectfully disagree and submit that the Examiner has failed in several aspects to make a *prima facie* case of obviousness.

A. Deshmukh Does Not Teach or Suggest Amphoteric Liposomes

Applicants reassert that a skilled artisan would not be motivated to create amphoteric liposomes in view of Deshmukh. The Examiner correctly recognizes that Deshmukh does not teach liposomes having an overall negative charge at physiological pH (Office Action at p. 6, ll. 10-13); however the Examiner further asserts that the artisan would be motivated to form negatively-charged liposomes. This latter assert is unsupported by Deshmukh. The Examiner refers to col. 7, ll. 30-35 in which Deshmukh suggests the further inclusion of negatively-charged lipids. Here, contrary to the Examiner's assertion, Deshmukh specifically directs that the liposomal charge remain positive. Deshmukh states: "The liposomes may also contain negatively charged lipids so long as the net charge of the complexes formed is positive." The complexes to which Deshmukh refers must be the charged lipid complexes that form the liposomes. Thus, Deshmukh directs that the net charge must remain positive even allowing for the inclusion of some negatively charged lipids.

Furthermore, negatively-charged liposomes would defeat a key advantage of the Deshmukh liposomes. Deshmukh requires cationic liposomes because the preferred biologically active substances to be loaded into the liposomes are negatively charged (e.g., nucleic acids, negatively charged proteins, carbohydrates including polysaccharides, and negatively charged drugs). Deshmukh at col. 8, ll. 48-51. In order to facilitate loading and/or adherence of the negatively charged active substances into/onto the Deshmukh liposomes, the Deshmukh liposomes must be cationic. It is difficult, if not impossible, to load anionic liposomes with anionic substances. This is yet further evidence that Deshmukh intended to limit the liposomes to cationic lipid mixtures, even if anionic lipids are present. For these reasons, Deshmukh cannot render obvious the presently claimed invention. This rejection should be withdrawn and such action is respectfully requested.

V. Rejection of Claims 3, 5, and 6

Claims 3, 5, and 6 are not rendered obvious by any combination of references cited above. Claim 3 (from which claims 5 and 6 depend) requires that the amphoteric liposomes

contain a lipid that carries both a positive and a negative charge (i.e., are amphoteric). The Examiner has not identified any reference that teaches or suggests using these amphoteric lipids in the preparation of liposomes. This rejection should be withdrawn and such action is respectfully requested.

VI. Rejection over Hafez in view of Deshmukh

Claims 5-11 and 22 stand rejected under 35 U.S.C. § 103(a) as obvious over Hafez in view of Deshmukh. The Examiner applies Hafez as disclosing liposomes containing cationic and anionic lipids. The Examiner notes that Hafez lacks a teaching to include a neutral lipid and an active agent. The Examiner asserts that it would have been obvious to combine the teachings of Hafez with that of Deshmukh which describes including a neutral lipid and an active agent such as DNA, RNA, or proteins to arrive at the presently claimed invention. Applicants respectfully traverse.

A. Hafez and Deshmukh Cannot Properly Be Combined

As discussed above and contrary to the Examiner's assertion, Deshmukh requires that liposomes formed according to his disclosure be cationic; although some anionic lipids are permitted provided that the net charge remains positive. Thus, the teachings of Hafez and Deshmukh cannot be properly combined because these references have different requirements that are mutually exclusive. Hafez teaches the creation of liposomes having a pH_f of 4 – 6.7 (i.e., that are anionic at physiological pH). Deshmukh requires cationic liposomes (col. 7, lines. 33-35 and col. 7, lines. 46-51). It is impossible for a liposome to be simultaneously anionic (satisfying Hafez) and cationic (satisfying Deshmukh) at physiological pH. The mutually exclusive requirements of these two references, therefore, renders the combination of references improper because there is no motivation to combine the otherwise incompatible teachings. The Examiner is "cherry-picking" the prior art for claim elements irrespective of their context. The Examiner improperly combines the teachings of two incompatible and mutually exclusive liposomal formulations in order to arrive at, what is asserted to be, the claimed invention. This is the type of hindsight analysis that the Federal Circuit strongly cautions against.

Accordingly, Applicants submit that the combination of Hafez and Deshmukh does not render the instant invention obvious. This rejection should be withdrawn and such action is respectfully requested.

VII. Liposomes of the Invention Have Unexpected Properties

The liposomes of the present invention have surprising and unexpected properties not predicted by the prior art. Applicants observed that the addition of neutral lipids to amphoteric liposomes significantly enhances transfection efficiency. Panzner Declaration, ¶¶ 15-18. Applicants have demonstrated this surprising effect using a variety of liposomal formulations. For example, in Experiment #1, Applicants formed liposomes from DOTAP, a strong cation, and oleic acid (OA), a weak anion. The addition of up to 20% of either POPC or DOPE, neutral lipids, significantly enhanced antisense transfection efficiency in a HeLa cell line. Panzner Declaration ¶¶ 15-16. This surprising effect was replicated using the weak cations MoChol, DOIM, and CHIM in combination with other weak anions including DOGS and DMGS. Panzner Declaration ¶ 17. Finally, a similar effect was observed with the addition of POPC and DOPE to liposomes comprising the amphoteric lipid HistChol. Panzner Declaration ¶ 18.

In sum, Applicants have surprisingly discovered that the addition of neutral lipids to amphoteric liposomes significantly enhances transfection efficiency. This effect was neither suggested nor expected from the teachings of Hafez alone or in combination with Huang, Lishko, or Deshmukh. Accordingly, in view of this surprising result, Applicants respectfully submit that the invention as currently claimed is unobvious and that this rejection should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. If the Examiner should have any questions concerning this communication or feels that an interview would be helpful to expedite allowance of this case, the Examiner is requested to call Applicants' undersigned attorney.

Respectfully submitted,

Dated: 10/18/06

Anthony C. Kuhlmann, Ph.D.
Reg. No. 57,147

Brown Rudnick Berlack Israels LLP
One Financial Center, Box IP
Boston, MA 02111
Tel: (617) 856-8349
Fax: (617) 856-8201
Email: ip@brownrudnick.com

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